bio-protocol Preprint

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In-vitro stimulation of epithelial cells

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An abbreviated version of this protocol was published in eLIFE in Dec 2021

SARS-CoV-2 spike protein induces inflammation via TLR2-dependent activation of the NF-κB pathway

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Detailed protocol

Detailed protocol of in-vitro stimulation of epithelial cells

1. HEK293T cells, Calu3 cells or A549 cells were cultured in MDEM plus 10% FBS and 1% penicillin/streptomycin.
2. Cells were seeded in a 12-well plate at a concentration of 500000 cells per well with 1 ml medium per well.
3. Cell culture plates were incubated at 37°C with 5% CO2.
4. At 80-90% confluency, culture medium was removed and replaced with fresh medium containing S protein (500ng/ml). Control wells were added with fresh medium without S protein.
5. Cell culture plates were incubated at 37°C with 5% CO2 for desired time (2, 4, 8, 12, 24h).
6. Culture supernatants were aspirated or collected in tubes, and 500 ml Trizol reagent was added into each well.
7. Cell lysates in Trizol were collected in microcentrifuge tubes and processed for RNA isolation.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Khan, S. and Zaki, H. (2022). In-vitro stimulation of epithelial cells. Bio-protocol Preprint. bio-protocol.org/prep2088.
2. Khan, S., Shafiei, M. S., Longoria, C., Schoggins, J. W., Savani, R. C. and Zaki, H.(2021). SARS-CoV-2 spike protein induces inflammation via TLR2-dependent activation of the NF-κB pathway. eLIFE. DOI: 10.7554/eLife.68563